PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference JHB/03463WO/JLH	**FOR FURTHER ACTION**	See Form PCT/IPEA/416
International application No. PCT/GB2004/004912	International filing date *(day/month/year)* 22.11.2004	Priority date *(day/month/year)* 20.11.2003
International Patent Classification (IPC) or national classification and IPC A61K38/09, A61K38/39, A61P15/08		
Applicant QUEEN MARY & WESTFIELD COLLEGE et al.		

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 8 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☒ *sent to the applicant and to the International Bureau)* a total of 4 sheets, as follows:
      ☒ sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      ☒ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.
   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing and/or tables related thereto, in computer readable form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).

4. This report contains indications relating to the following items:

☒	Box No. I	Basis of the opinion
☐	Box No. II	Priority
☒	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
☐	Box No. IV	Lack of unity of invention
☒	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
☐	Box No. VI	Certain documents cited
☐	Box No. VII	Certain defects in the international application
☒	Box No. VIII	Certain observations on the international application

Date of submission of the demand	Date of completion of this report
20.06.2005	01.03.2006
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized Officer Bochelen, D Telephone No. +49 89 2399-8150

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/GB2004/004912

Box No. I Basis of the report

1. With regard to the **language**, this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This report is based on translations from the original language into the following language , which is the language of a translation furnished for the purposes of:
   - ☐ international search (under Rules 12.3 and 23.1(b))
   - ☐ publication of the international application (under Rule 12.4)
   - ☐ international preliminary examination (under Rules 55.2 and/or 55.3)

2. With regard to the **elements*** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

Description, Pages

1-4, 6-16	as originally filed
5	filed with telefax on 20.06.2005

Claims, Numbers

10-19	filed with telefax on 20.06.2005
1-9	received on 24.08.2005 with letter of 22.08.2005

Drawings, Sheets

1/6-6/6	as originally filed

☐ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing

3. ☐ The amendments have resulted in the cancellation of:
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

4. ☒ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).
   - ☒ the description, pages 5
   - ☒ the claims, Nos. 1-3,21
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

* *If item 4 applies, some or all of these sheets may be marked "superseded."*

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/GB2004/004912

Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application,

☒ claims Nos. 1-7 with regard to industrial applicabilty

because:

☒ the said international application, or the said claims Nos. 1-7 relate to the following subject matter which does not require an international preliminary examination (specify):

see separate sheet

☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. are so unclear that no meaningful opinion could be formed *(specify)*:

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☐ no international search report has been established for the said claims Nos.

☐ the nucleotide and/or amino acid sequence listing does not comply with the standard provided for in Annex C of the Administrative Instructions in that:

the written form	☐	has not been furnished
	☐	does not comply with the standard
the computer readable form	☐	has not been furnished
	☐	does not comply with the standard

☐ the tables related to the nucleotide and/or amino acid sequence listing, if in computer readable form only, do not comply with the technical requirements provided for in Annex C-*bis* of the Administrative Instructions.

☐ See separate sheet for further details

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/GB2004/004912

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-7,9-16,19
	No:	Claims	8,17-18
Inventive step (IS)	Yes:	Claims	1-7,11-16
	No:	Claims	8-10,17-19
Industrial applicability (IA)	Yes:	Claims	8-19
	No:	Claims	

2. Citations and explanations (Rule 70.7):

see separate sheet

Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

Re Item I
Basis of the report

1. The amendments filed with the telefax dated 20.06.05 introduce subject-matter which extends beyond the content of the application as filed, contrary to Article 34(2)(b) PCT. The amendments concerned are the following:

   the term "subsequent" in claim 1 is not supported in the original application,

   the feature "in an aqueous buffer" in claim 21 is not supported in the original application,

   the introduction of the feature "salt or analog" in claims 2-3 is a broadening of the scope of the application since the passage supporting this feature refers to the related peptides disclosed in WO95/32725

   the definition of the tripeptide RGD which is amended on page 5 of the description is not supported in the original application.

Re Item III
Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

2. Claims 1-7 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(I) PCT).

Re Item V
Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

D1: WO 01/13932 A (KING'S COLLEGE LONDON; FRASER, LYNN, REPSIS) 1 March 2001 (2001-03-01)
D2: WO 95/32725 A (QUEEN MARY AND WESTFIELD COLLEGE; VINSON, GAVIN, PAUL) 7 December 1995 (1995-12-07)
D3: VINSON G P ET AL: "ANGIOTENSIN II STIMULATES SPERM MOTILITY"

REGULATORY PEPTIDES, ELSEVIER SCIENCE BV, NL, vol. 67, 1996, pages 131-135, XP000979550 ISSN: 0167-0115

D4: FRASER L R ET AL: "Calcitonin, angiotensin II and FPP significantly modulate mouse sperm function." MOLECULAR HUMAN REPRODUCTION. MAR 2001, vol. 7, no. 3, March 2001 (2001-03), pages 245-253, XP002319525 ISSN: 1360-9947

D5: WENNEMUTH G ET AL: "Influence of fibronectin on the motility of human spermatozoa" INTERNATIONAL JOURNAL OF ANDROLOGY, vol. 20, no. 1, 1997, pages 10-16, XP009044560 ISSN: 0105-6263

D6: US-A-5 389 519 (BRONSON ET AL) 14 February 1995 (1995-02-14)

D7: FUSI F M ET AL: "Sperm surface fibronectin. Expression following capacitation." JOURNAL OF ANDROLOGY. 1992 JAN-FEB, vol. 13, no. 1, January 1992 (1992-01), pages 28-35, XP009044553 ISSN: 0196-3635

If not indicated otherwise the relevant passages are those mentioned in the search report.

Document D1 discloses the use of angiotensin II for stimulating the motility and capacitation of sperm.

Document D2 discloses the use of angiotensin II for increasing sperm motility and promoting fertilization of eggs.

Document D3 discloses that angiotensin II promotes sperm motility.

Document D4 discloses that angiotensin II promotes fertilization capacities of sperm.

Document D5 discloses that the addition of fibronectin to a sperm suspension decreases spermatozoa motility.

Document D6 discloses a BWW medium comprising fibronectin or vitronectin and increased expression of fibronectin at the surface of capacitated spermatozoa.

Document D7 discloses that the expression of fibronectin is increased at the surface of capacitated spermatozoa.

3. Novelty (Art. 33(1) and (2) PCT):

3.1 Document D5 does not explicitly disclose that the sperm is conserved, however D5 discloses that fibronectin decreases and even suppresses the motility of spermatozoa. There is no reason to assume that in D5 the decrease of motility of the spermatozoa after fibronectin treatment would be due to the death of the spermatozoa. Therefore, it is considered that claim 8 is anticipated by document D5.

3.2 It is stressed that the terms "reproduction cell medium" and "sperm inhibition composition" are not characterizing. Document D5 discloses fibronectin in phosphate buffered saline (PBS) see page 11 right column 1st§. Document D6 discloses BWW solutions with fibronectin or vitronectin(see col. 11 ex 10). Claim 17 lacks thus novelty in the sense of Art. 33(2) PCT.

4. Inventive step (Art. 33(1) and (3) PCT):

4.1 Document D2 which is considered to be the closest prior art discloses a method for increasing sperm motility and promoting fertilization of eggs by using angiotensin II. The present claims 1 and 11 differs in that the sperm has been put in a low motility non-capacitated state with an extracellular matrix before capacitation by angiotensin II. The problem to be solved may be regarded as to provide a method for bringing spermatozoa in a low motility non-capacitated state before capacitation by angiotensin II. Document D5 discloses that fibronectin decreases spermatozoa motility (see D5: p14 col1 §1). There is no indication in the prior art which would prompt a skilled man to combine both techniques: an extracellular protein to bring spermatozoa in a non-capacitated state and angiotensin II for capacitating the sperm. Therefore, an inventive step is acknowledged for claims 1-7 and 11-16.

4.2 Dependent claims 9 and 10 are obvious in view of D5.

4.3 Claim 19 is an obvious alternative galenic form of the extracellular matrix protein disclosed in D5 and D6. Said claim is not inventive in the sense of Art. 33 (3) PCT.

5. Industrial applicability (Art. 33 (1) and (4) PCT):

For the assessment of the present claims 1-7 on the question whether they are industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

Re Item VIII

Certain observations on the international application

6. Claims 1 and 11 are not clear since it is not clear which compounds fall in the scope of the terms "related peptides" (Art. 6 PCT).

The extracellular matrix proteins are capable of binding to cell surfaces and this is believed to be involved in maintaining sperm in a low motility, non-capacitated state in accordance with this invention, although the actual mechanism is not yet known. Suitable extracellular matrix proteins for use in the present invention include fibronectin, vitronectin and laminin.

As a capacitating agent, angiotensin II can be replaced by related peptides such as the salts and analogs mentioned in WO 95/32725, the entire disclosure of which is incorporated herein by reference. A suitable analog is angiotensin II amide.

Alternatively peptides containing the tripeptide motif RGD (Arg-Gly-Asp) may be used as agents for capacitation. This motif may be provided by the tripeptide RGD itself or by other small peptides, such as the commercially available tetrapeptide RGDS (Arg-Gly-Asp-Ser). RGD is a tripeptide motif found in ~~all the extracellular matrix proteins, to which~~ to which all the extracellular proteins the integrins in the cell membrane bind. Use of free RGD, or other peptides containing the motif RGD, therefore competes with the extracellular matrix proteins, and will inhibit cell binding by the proteins. Effective amounts can be found by routine testing using the Examples below for guidance.

RGD is suitably used in combination with angiotensin II, because the RGD inhibits extracellular matrix protein binding, increasing the effectiveness of added angiotensin II in stimulating motility and hence capacitation/activation. However angiotensin II is still an effective capacitation agent in the absence of RGD peptides.

For storage, fresh sperm is preferably added to a sperm extender medium or other reproductive cell medium.

Accordingly a further aspect of the invention is a sperm extender medium containing an extracellular matrix protein such as fibronectin, vitronectin or laminin, so that added sperm is maintained in a non-capacitated state. Sperm extender media are commercially available and typically comprise a buffered aqueous solution.

CLAIMS

1. A sperm regulation method which comprises adding an extracellular matrix protein to a sperm sample to bring the sperm into a non-capacitated state, and subsequently adding angiotensin II or a related peptide to stimulate motility and capacitate the sperm.

2. A method according to claim 1, in which the sperm sample containing an extracellular matrix protein has been stored prior to adding angiotensin II or a salt or analog to capacitate the sperm.

3. A method according to claim 2 in which the sperm sample containing an extracellular matrix protein has been frozen or chilled for storage and is thawed prior to adding angiotensin II or a salt or analog to capacitate the sperm.

4. A method according to any one of claims 1 to 3 which is carried out *in vitro*.

5. A method according to any one of claims 1 to 3 which is carried out at least partly *in vivo*.

6. A method according to any one of claims 1 to 5 in which the extracellular matrix protein is selected from fibronectin, vitronectin and laminin.

7. A method according to any one of claims 1 to 6 in which the capacitating agent is angiotensin II or angiotensin II amide.

8. Use of one or more extracellular matrix proteins as an agent to conserve sperm in a low motility non-capacitated state.

9. Use according to claim 8 in which the extracellular matrix protein-containing sperm is stored in liquid form.

10. Use according to claim 9 in which the extracellular matrix protein-containing sperm is stored in frozen form.

11. Use of angiotensin II or related peptides as an agent for stimulating motility and capacitation of sperm samples that have been conserved in a low motility non-capacitated state by addition of an extracellular matrix protein.

12. Use according to claim 11 in which the sperm sample that has been conserved in a non-capacitated state is obtained by thawing extracellular matrix protein-containing frozen sperm.

13. Use according to claim 12 in which the sperm sample that has been conserved in a non-capacitated state is an extracellular matrix protein-containing sperm sample that has been stored in liquid form.

14. Use according to claim 13 in which the sperm sample that has been conserved in a non-capacitated state is fresh sperm in which capacitation has been suppressed until ready for use.

15. Use according to any one of claims 11 to 14 in which the extracellular matrix protein is selected from fibronectin, vitronectin and laminin.

16. Use according to any one of claims 11 to 15 in which the capacitating agent is angiotensin II or angiotensin II amide.

17. A reproduction cell medium comprising one or more extracellular matrix proteins as an agent to conserve sperm in a non-capacitated state in an aqueous buffer.

18. A reproduction cell medium according to claim 17 in which the extracellular matrix protein is selected from fibronectin, vitronectin and laminin.

19. A sperm inhibition composition comprising one or more extracellular matrix proteins dispersed in a gel, cream or pessary base.